

A noncovalent interaction between two molecules that has very slow dissociation kinetics can also function as a crosslink. For example, reactive derivatives of phospholipids can be used to link the liposomes or cell membranes in which they are incorporated to antibodies or enzymes. Biotinylation and haptenylation reagents (Chapter 4) can also be thought of as heterobifunctional crosslinking reagents because they comprise a chemically reac-

tive group as well as a biotin or hapten moiety that binds with high affinity to avidin or an anti-hapten antibody, respectively. Similarly, avidin, streptavidin, NeutrAvidin biotin-binding protein and CaptAvidin biotin-binding protein (Section 7.5) can tightly bind up to four molecules of a biotinylated target, and immunoglobulin G (IgG) can bind up to two haptens.

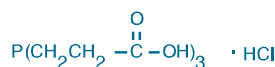


Figure 5.2 T-2556 tris-(2-carboxyethyl)phosphine, hydrochloride.

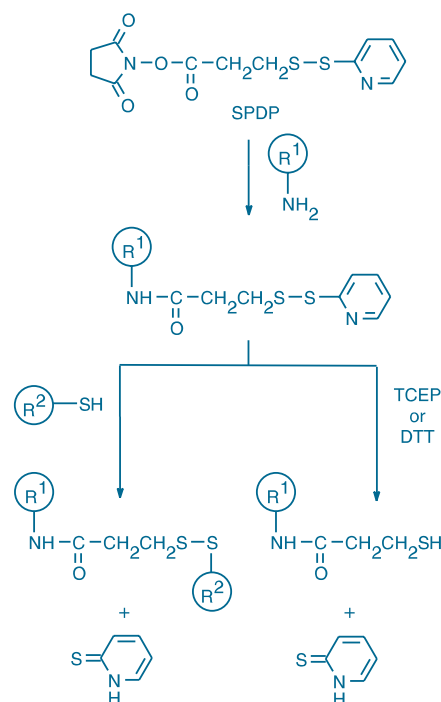


Figure 5.3 SPDP derivatization reactions. SPDP (S-1531) reacts with an amine-containing biomolecule at pH 7 to 9, yielding a mixed disulfide. The mixed disulfide can then be reacted with a reducing agent such as DTT (D-1532) or TCEP (T-2556) to release a pyridylthiopropionyl conjugate or with a thiol-containing biomolecule to form a disulfide-linked biomolecule pair. Either reaction can be quantitated by measuring the amount of 2-pyridylthione chromophore released during the reaction.

We find that the combination of SPDP and SMCC labeling is usually most reliable for forming stable protein-protein chemical crosslinks.

5.2 Chemical Crosslinking Reagents

The most common schemes for forming a heteroconjugate involve the indirect coupling of an amine group on one biomolecule to a thiol group on a second biomolecule, usually by a two- or three-step reaction sequence. The high reactivity of thiols (Chapter 2) and — with the exception of a few proteins such as β -galactosidase — their relative rarity in most biomolecules make thiol groups ideal targets for controlled chemical crosslinking. If neither molecule contains a thiol group, then one or more can be introduced using one of several thiolation methods. The thiol-containing biomolecule is then reacted with an amine-containing biomolecule using a heterobifunctional crosslinking reagent such as one of those described in Amine–Thiol Crosslinking, below.

Thiolation of Biomolecules

Introducing Thiol Groups

Several methods are available for introducing thiols into biomolecules, including the reduction of intrinsic disulfides, as well as the conversion of amine, aldehyde or carboxylic acid groups to thiol groups:

- Disulfide crosslinks of cystines in proteins can be reduced to cysteine residues by dithiothreitol¹ (DTT, D-1532), tris-(2-carboxyethyl)phosphine (TCEP, T-2556; Figure 5.2) or tris-(2-cyanoethyl)phosphine (T-6052). However, reduction may result in loss of protein activity or specificity. Excess DTT must be carefully removed under conditions that prevent reformation of the disulfide,² whereas excess TCEP usually does not need to be removed before carrying out the crosslinking reaction. TCEP is also stable at higher pH values than is the air-sensitive DTT reagent.
- Amines can be indirectly thiolated by reaction with succinimidyl 3-(2-pyridylthio)propionate³ (SPDP, S-1531), followed by reduction of the 3-(2-pyridylthio)propionyl conjugate with DTT or TCEP (Figure 5.3). Reduction releases the 2-pyridylthione chromophore, which can be used to determine the degree of thiolation.
- Amines can be indirectly thiolated by reaction with succinimidyl acetylthioacetate⁴ (SATA, S-1553), followed by removal of the acetyl group with 50 mM hydroxylamine or hydrazine at near-neutral pH (Figure 5.1). This reagent is most useful when disulfides are essential for activity, as is the case for some peptide toxins.
- Thiols can be incorporated at carboxylic acid groups by an EDAC-mediated reaction with cystamine, followed by reduction of the disulfide with DTT or TCEP;^{5,6} see Amine–Carboxylic Acid and Thiol–Carboxylic Acid Crosslinking below.
- Tryptophan residues in thiol-free proteins can be oxidized to mercaptotryptophan residues, which can then be modified by iodoacetamides or maleimides.^{7–9}

Our preferred reagent combination for protein thiolation is SPDP/DTT or SPDP/TCEP.¹⁰ Molecular Probes uses SPDP to prepare a reactive R-phycoerythrin derivative (P-806, Section 6.4), providing researchers with the optimal number of pyridylthio groups for crosslinking the phycobiliprotein to thiolated antibodies, enzymes and other biomolecules through disulfide linkages.¹¹ More commonly, the pyridylthio groups are first reduced to thiols, which are then reacted with maleimide- or iodoacetamide-derivatized proteins (Figure 5.3, Figure 5.4). SPDP can also be used to thiolate oligonucleotides¹² and — like all of the thiolation reagents in this section — to introduce the

highly reactive thiol group into peptides, onto cell surfaces or onto affinity matrices for subsequent reaction with fluorescent, enzyme-coupled or other thiol-reactive reagents. In addition, because the 3-(2-pyridyl)dithio)propionyl conjugate releases the 2-pyridinethione chromophore upon reduction, SPDP is useful for quantitating the number of reactive amines in an affinity matrix.¹³

Measuring Thiolation of Biomolecules

To ensure success in forming heterocrosslinks, it is important to know that a molecule has the proper degree of thiolation. We generally find that two to three thiol residues per protein are optimal. Following removal of excess reagents, the degree of thiolation in proteins or other molecules thiolated with SPDP can be directly determined by measuring release of the 2-pyridinethione chromophore³ ($\epsilon_{343\text{ nm}} \sim 8000\text{ cm}^{-1}\text{M}^{-1}$).

Alternatively, the degree of thiolation and presence of residual thiols in a solution can be assessed using 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB, Ellman's reagent; D-8451), which stoichiometrically yields the chromophore 5-mercapto-2-nitrobenzoic acid ($\epsilon_{410\text{ nm}} \sim 13,600\text{ cm}^{-1}\text{M}^{-1}$) upon reaction with a thiol group.^{14,15} DTNB can also be used to quantitate residual phosphines, including TCEP;¹⁶ in this case, two molecules of 5-mercapto-2-nitrobenzoic acid are formed per reaction with one molecule of a phosphine.

Thiol and Sulfide Quantitation Kit

Ultrasensitive colorimetric quantitation of both protein and nonprotein thiols is now possible using our Thiol and Sulfide Quantitation Kit (T-6060). In this assay, which is based on a method reported by Singh,^{17,18} thiols reduce a disulfide-inhibited derivative of papain, stoichiometrically releasing the active enzyme. Activity of the enzyme is then measured using the chromogenic papain substrate L-BAPNA (Figure 5.5). Although thiols can also be quantitated using DTNB (Ellman's reagent, see above), the enzymatic amplification step in our quantitation kit enables researchers to detect as little as 0.2 nanomoles of a thiol — a sensitivity that is about 100-fold better than that achieved with DTNB. Thiols in proteins and potentially in other high molecular weight molecules can

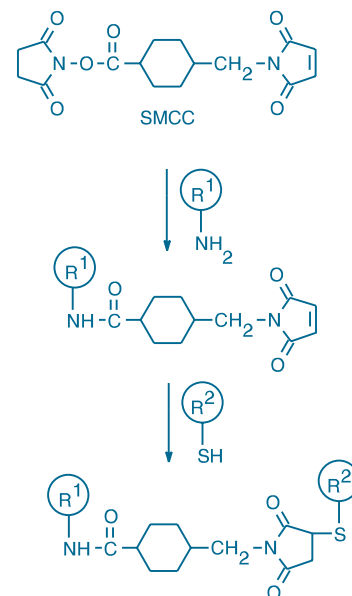


Figure 5.4 Two-step reaction sequence for cross-linking biomolecules using the heterobifunctional crosslinker SMCC (S-1534).

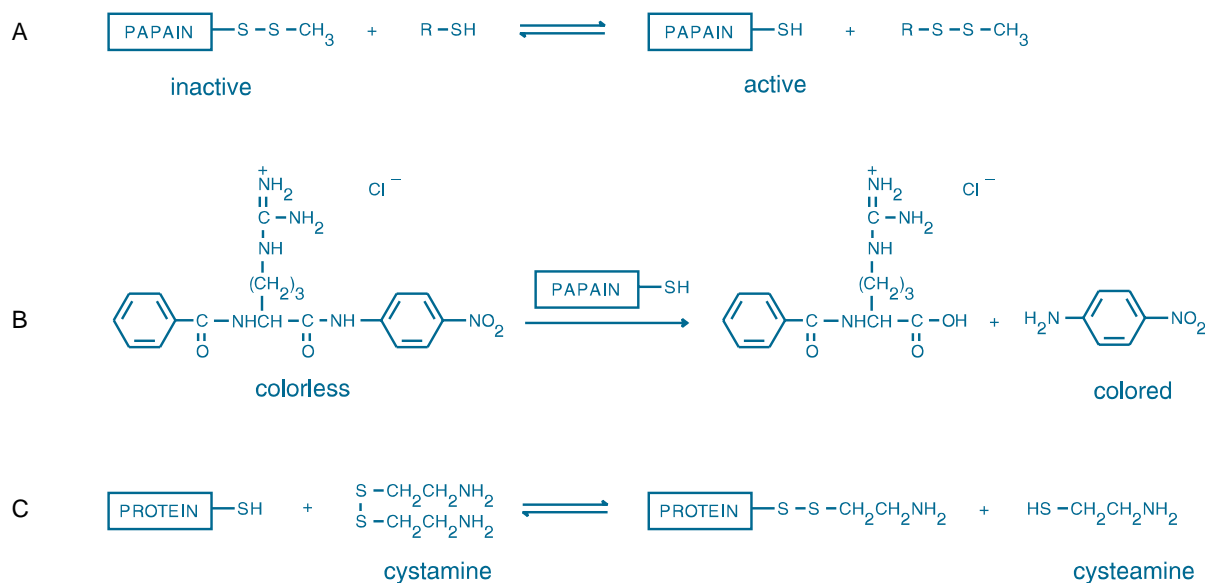


Figure 5.5 Chemical basis for thiol detection using the Thiol and Sulfide Quantitation Kit (T-6060): A) the inactive disulfide derivative of papain, papain-SSCH₃, is activated in the presence of thiols; B) active papain

cleaves the substrate L-BAPNA, releasing the *p*-nitroaniline chromophore; C) protein thiols, often poorly accessible, exchange with cystamine to generate 2-mercaptoethylamine (cystamine), which is easily detected.

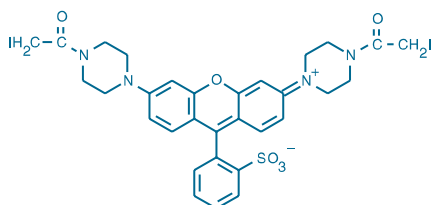


Figure 5.6 B-10621 bis-((*N*-iodoacetyl)piperazinyl)sulfonerhodamine.

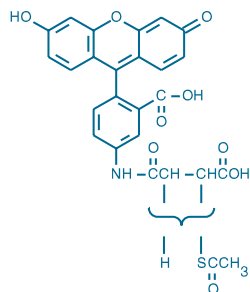
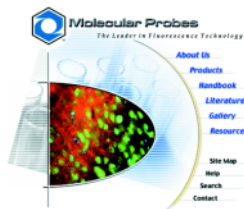


Figure 5.7 A-685 5-((2-(and-3)-*S*-(acetylmercapto)succinyl)amino)fluorescein.



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be detected indirectly by incorporating the disulfide cystamine into the solution. Cystamine undergoes an exchange reaction with protein thiols, yielding 2-mercaptoethylamine (cysteamine), which then releases active papain. All traces of reducing agents must be removed before determining free thiols in proteins. The Thiol and Sulfide Quantitation Kit contains:

- Papain-SSCH₃, the disulfide-inhibited papain derivative
- L-BAPNA, a chromogenic papain substrate
- DTNB (Ellman's reagent), for calibrating the assay
- Cystamine
- L-Cysteine, a thiol standard
- Buffer
- A protocol for measuring thiols, inorganic sulfides and maleimides

Sufficient reagents are provided for approximately 50 assays using 1 mL assay volumes and standard cuvettes or 250 assays using a microplate format. This kit can also be used to detect phosphines, sulfites and cyanides, with detection limits of about 0.5, 1 and 5 nanomoles, respectively.

Thiol–Thiol Crosslinking

Oxidation

Thiol residues in close proximity can be oxidized to disulfides by either an intra- or intermolecular reaction. In many circumstances, however, this oxidation reaction is reversible and difficult to control.

Fluorescent Thiol–Thiol Crosslinkers

Dibromobimane (bBBR, D-1379) is an interesting crosslinking reagent for proteins because it is unlikely to fluoresce until both of its alkylating groups have reacted.¹⁹ It has been used to crosslink thiols in myosin,²⁰ actin,²¹ hemoglobin,²² *Escherichia coli* lactose permease²³ and mitochondrial ATPase.²⁴ It has also been shown to intramolecularly crosslink thiols in a complex of nebulin and calmodulin.²⁵ In addition, dibromobimane has been used to probe for the proximity of dual-cysteine mutagenesis sites in ArSA ATPase²⁶ and P-glycoprotein.^{27–29}

The thiol-reactive homobifunctional crosslinker bis-((*N*-iodoacetyl)piperazinyl)sulfonerhodamine (B-10621) is derived from a relatively rigid rhodamine dye (Figure 5.6). This crosslinker, which is similar to a thiol-reactive rhodamine-based crosslinking reagent that was used to label regulatory light-chains of chicken gizzard myosin for fluorescence polarization experiments,³⁰ may be useful for proximity studies and for fluorescence polarization measurements.

Amine–Amine Crosslinking

The scientific literature contains numerous references to reagents that form crosslinks between amines of biopolymers. Homobifunctional amine crosslinkers include glutaraldehyde, bis(imido esters), bis(succinimidyl esters), diisocyanates and diacid chlorides.³¹ However, these reagents tend to yield high molecular weight aggregates, making them unsuitable for preparing conjugates between two different amine-containing biomolecules. Such conjugates are more commonly prepared by thiolating one or more amines on one of the biomolecules and converting one or more amines on the second biomolecule to a thiol-reactive functional group such as a maleimide or iodoacetamide, as described below in Amine–Thiol Crosslinking.

Direct amine–amine crosslinking routinely occurs during fixation of proteins, cells and tissues with formaldehyde or glutaraldehyde. These common aldehyde-based fixatives are also used to crosslink amine and hydrazine derivatives to proteins and other amine-containing polymers. For example, lucifer yellow CH (L-453, Section 14.3) is nonspecifically conjugated to surrounding biomolecules by aldehyde-based fixatives in order to preserve the dye's staining pattern during subsequent tissue manipulations.³² Also, biotin hydrazides (Section 4.2) have been directly coupled to nucleic acids with

glutaraldehyde,^{33,34} a reaction that is potentially useful for conjugating fluorescent hydrazides to DNA.

Glutaraldehyde is still used by some companies and research laboratories to couple horseradish peroxidase, which has only six lysine residues,³⁵ to proteins with a larger numbers of lysine residues. However, this practice can result in variable molecular weights and batch-to-batch inconsistency. Consequently, we prepare our horseradish peroxidase conjugates (Section 7.3, Section 7.6) using SPDP- and SMCC-mediated reactions (Figure 5.3, Figure 5.4).

Amine–Thiol Crosslinking

Indirect crosslinking of the amines in one biomolecule to the thiols in a second biomolecule is the predominant method for forming a heteroconjugate. If one of the biomolecules does not already contain one or more thiol groups, it is necessary to introduce them using one of the thiolation procedures described above in Thiolation of Biomolecules. Thiol-reactive groups such as maleimides or iodoacetamides are typically introduced into the second biomolecule by modifying a few of its amines with a heterobifunctional crosslinker containing both a succinimidyl ester and either a maleimide or an iodoacetamide. The maleimide- or iodoacetamide-modified biomolecule is then reacted with the thiol-containing biomolecule to form a stable thioether cross-link. Chromatographic methods are usually employed to separate the higher molecular weight heteroconjugate from the unconjugated biomolecules.

Introducing Maleimides at Amines

Succinimidyl *trans*-4-(maleimidylmethyl)cyclohexane-1-carboxylate³⁶ (SMCC, S-1534) is our reagent of choice for introducing thiol-reactive groups at amine sites because of the superior chemical stability of its maleimide and its ease of use³⁷ (Figure 5.4).

Introducing Disulfides at Amines

Our preferred method for preparing heteroconjugates employs the thiolation reagent SPDP (S-1531). The pyridyldisulfide intermediate that is initially formed by reaction of SPDP with amines can form an unsymmetrical disulfide through reaction with a second thiol-containing molecule^{3,11} (Figure 5.3). The thiol-containing target can be a molecule such as β -galactosidase that contains intrinsic thiols or a molecule in which thiols have been introduced using one of the thiolation procedures described above in Thiolation of Biomolecules. In either case, it is essential that all reducing agents, such as DTT and TCEP, are absent. The heteroconjugate's disulfide bond is about as stable and resistant to reduction as those found in proteins; it can be reduced with DTT or TCEP to generate two thiol-containing biomolecules.

Protein–Protein Crosslinking Kit

Our Protein–Protein Crosslinking Kit (P-6305) provides all of the reagents and purification media required to perform three protein–protein conjugations in which neither protein contains thiol residues. The chemistry used to thiolate the amines of one of the proteins with SPDP and to convert the amines of the second protein to thiol-reactive maleimides with SMCC is shown in Figure 5.3 and Figure 5.4, respectively. Included in the kit are:

- SPDP, for thiolating amines
- SMCC, for converting amines to thiol-reactive maleimides
- TCEP, for reducing the pyridyldisulfide intermediate
- *N*-ethylmaleimide (NEM), for capping residual thiols
- Six reaction tubes, each containing a magnetic stir bar
- Spin columns plus collection tubes
- Dimethylsulfoxide (DMSO)
- A detailed crosslinking protocol

The Protein–Protein Crosslinking Kit was designed to prepare and purify protein–protein conjugates; however, it can be readily modified for generating peptide–protein or enzyme–nucleic acid conjugates or for conjugating biomolecules to affinity matrices.

Molecular Probes has considerable experience in preparing protein–protein conjugates and will apply this expertise to a researcher's particular application through our custom synthesis service. We provide custom conjugation services on an exclusive or nondisclosure basis when requested. For more information or a quote, please contact our Custom and Bulk Sales Department.

Assaying Maleimide- and Iodoacetamide-Modified Biomolecules

The potential instability of maleimide derivatives and the photosensitivity of iodoacetamide derivatives may make it advisable to assay the modified biomolecule for thiol reactivity before conjugation with a thiol-containing biomolecule. Fluorescein SAMSA (A-685, Figure 5.7), which is our only fluorescent reagent that can generate a free thiol group, was designed for assaying whether a biomolecule is adequately labeled with a heterobifunctional maleimide or iodoacetamide crosslinker. Brief treatment of fluorescein SAMSA with NaOH at pH 10 liberates a free thiol. By adding base-treated fluorescein SAMSA to a small aliquot of the crosslinker-modified biomolecule, the researcher can check to see whether the biomolecule has been sufficiently labeled before proceeding to the next step. The degree of modification can be approximated from either the absorbance or the fluorescence of the conjugate following quick purification on a gel-filtration column.

Alternatively, thiol reactivity of the modified biomolecule can be assayed using the reagents provided in our Thiol and Sulfide Quantitation Kit (T-6060), a product that is described above.^{17,18} Once unconjugated reagents have been removed, a small aliquot of the maleimide- or iodoacetamide-modified biomolecule can be reacted with excess cysteine. Thiol-reactive groups can then be quantitated by determining the amount of cysteine consumed in this reaction with the Thiol and Sulfide Quantitation Kit.

Amine–Carboxylic Acid and Thiol–Carboxylic Acid Crosslinking

1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC, E-2247) can react with biomolecules to form “zero-length” crosslinks, usually within a molecule or between subunits of a protein complex. In this chemistry, the crosslinking reagent is not incorporated into the final product. The water-soluble carbodiimide EDAC crosslinks a specific amine and carboxylic acid between subunits of allophycocyanin, thereby stabilizing its assembly.³⁸ Molecular Probes uses EDAC to stabilize allophycocyanin in its

allophycocyanin conjugates (Section 6.4). EDAC has also been used to form intramolecular crosslinks in myosin subfragment-1,³⁹ intermolecular crosslinks in actomyosin,⁴⁰ intersubunit crosslinks of chloroplast subunits⁴¹ and crosslinks between proteins and DNA.⁴² Addition of *N*-hydroxysuccinimide or *N*-hydroxysulfosuccinimide (NHSS, H-2249) is reported to enhance the yield of carbodiimide-mediated conjugations,⁴³ indicating the *in situ* formation of a succinimidyl ester-activated protein. EDAC has been reported to be impermeant to cell membranes,⁴⁴ which should permit selective surface labeling of cellular carboxylic acids with fluorescent amines.

Reaction of carboxylic acids with cystamine (NH₂CH₂CH₂S-SCH₂CH₂NH₂) and EDAC followed by reduction with DTT results in thiolation at carboxylic acids.⁶ This indirect route to amine-carboxylic acid coupling is particularly suited to acidic proteins with few amines, carbohydrate polymers,⁵ heparin, poly(glutamic acid) and synthetic polymers lacking amines. The thiolated biomolecules can also be reacted with any of the probes described in Chapter 2.

Crosslinking Amines to Acrylamide Polymers

The succinimidyl ester of 6-((acryloyl)amino)hexanoic acid (acryloyl-X, SE; A-20770) reacts with amines of proteins, amine-modified nucleic acids and other biomolecules to yield acrylamides that can be copolymerized into polyacrylamide matrices or on surfaces, such as in microarrays and in biosensors. For example, streptavidin acrylamide (S-21379, Section 7.6) copolymerizes with acrylamide on polymeric surfaces to create a uniform monolayer of the immobilized protein. The streptavidin can then bind biotinylated ligands, including biotinylated hybridization probes, enzymes, antibodies and drugs.⁴⁵

Crosslinking Liposomes and Cell Membranes to Biomolecules

All of the chemical crosslinkers described above form covalent bonds with their targets. However, reagents used to crosslink

liposomes, cell membranes and potentially other lipid assemblies to biomolecules typically comprise a phospholipid derivative to anchor one end of the crosslink in the lipid layer and a reactive group at the other end to attach the membrane assembly to the target biomolecule.

Chemically Reactive and Biotinylated Phospholipids

Molecular Probes offers a maleimide-containing phospholipid (MMCC DHPE, M-1618) that can be incorporated into liposomes, then coupled to thiolated antibodies,⁴⁶ streptavidin,⁴⁷ lectins⁴⁸ and other proteins.^{49,50} Similarly, our phospholipid derivatives of biotin and biotin-X (B-1550, B-1616) can be used to prepare liposomes that have high affinity for avidin conjugates.⁵¹⁻⁵³

Applications for Liposome Bioconjugates

Liposome bioconjugates are versatile reagents that can serve as a means of targeted delivery — either of the contents of the liposome's aqueous cavity or of the components in its lipid membrane — to a particular site recognized by its biomolecule tag. Representative applications include:

- Following receptor-mediated endocytosis of liposomes by flow cytometry⁵⁴
- Loading liposomes with fluorescent dyes, including any of the polar tracers described in Section 14.3, for amplified detection in imaging and flow cytometry⁵⁵⁻⁵⁸
- Measuring anti-protein antibody using antigen-bearing liposomes in a liposome immune-lysis assay (LILA)⁵⁹
- Studying lateral and structural organization at aqueous interfaces⁶⁰⁻⁶³
- Targeting delivery of enzyme inhibitors⁶⁴ and oligodeoxyribonucleotides⁶⁵ into cells

References

1. *Bioconjug Chem* 12, 421 (2001); 2. *Methods Enzymol* 143, 246 (1987); 3. *Biochem J* 173, 723 (1978); 4. *Anal Biochem* 132, 68 (1983); 5. *Biosci Biotechnol Biochem* 61, 1836 (1997); 6. *Biochim Biophys Acta* 1038, 382 (1990); 7. *Biochim Biophys Acta* 971, 307 (1988); 8. *Biochim Biophys Acta* 971, 298 (1988); 9. *J Biol Chem* 255, 10884 (1980); 10. *Methods Mol Biol* 45, 235 (1995); 11. *J Cell Biol* 93, 981 (1982); 12. *Nucleic Acids Res* 17, 4404 (1989); 13. *J Biochem Biophys Methods* 12, 349 (1986); 14. *Methods Enzymol* 233, 380 (1994); 15. *Methods Enzymol* 91, 49 (1983); 16. *Anal Biochem* 220, 5 (1994); 17. *Bioconjug Chem* 5, 348 (1994); 18. *Anal Biochem* 213, 49 (1993); 19. *Anal Biochem* 225, 174 (1995); 20. *Proc Natl Acad Sci U S A* 97, 1461 (2000); 21. *J Mol Biol* 299, 421 (2000); 22. *Biochim Biophys Acta* 622, 201 (1980); 23. *Proc Natl Acad Sci U S A* 93, 10123 (1996); 24. *FEBS Lett* 150, 207 (1982); 25. *Biochemistry* 40, 7903 (2001); 26. *J Biol Chem* 271, 24465 (1996); 27. *J Biol Chem* 275, 39272 (2000); 28. *J Biol Chem* 274, 35388 (1999); 29. *Kidney Int* 51, 1797 (1997); 30. *Bioconjug Chem* 9, 160 (1998); 31. *Methods Enzymol* 172, 584 (1989); 32. *Nature* 292, 17 (1981); 33. *Nucleic Acids Res* 17, 4899 (1989); 34. *Chem Pharm Bull (Tokyo)* 37, 1831 (1989); 35. *Eur J Biochem* 96, 483 (1979); 36. *Eur J Biochem* 101, 395 (1979); 37. *Anal Biochem* 198, 75 (1991); 38. *Cytometry* 8, 91 (1987); 39. *Biochemistry* 33, 6867 (1994); 40. *Biophys J* 68, 35 (1995); 41. *Biochim Biophys Acta* 1101, 97 (1992); 42. *J Mol Biol* 123, 149 (1978); 43. *Anal Biochem* 156, 220 (1986); 44. *J Biol Chem* 275, 977 (2000); 45. *Anal Biochem* 282, 200 (2000); 46. *Biol Cell* 47, 111 (1983); 47. *Anal Biochem* 207, 341 (1992); 48. *Cell Biol Int Rep* 9, 1123 (1985); 49. *J Immunol Methods* 132, 25 (1990); 50. *Methods Enzymol* 149, 111 (1987); 51. *Biophys J* 75, 2352 (1998); 52. *Biophys J* 70, 57 (1996); 53. *Methods Enzymol* 149, 119 (1987); 54. *Biochem J* 214, 189 (1983); 55. *J Fluorescence* 3, 33 (1993); 56. *J Immunol Methods* 121, 1 (1989); 57. *Cytometry* 8, 562 (1987); 58. *J Immunol Methods* 100, 59 (1987); 59. *J Immunol Methods* 75, 351 (1984); 60. *Biophys J* 68, 312 (1995); 61. *Biophys J* 66, 305 (1994); 62. *Biophys J* 65, 2160 (1993); 63. *J Membr Biol* 135, 83 (1993); 64. *J Cell Biol* 102, 1630 (1986); 65. *Proc Natl Acad Sci U S A* 87, 2448 (1990).

Data Table — 5.2 Chemical Crosslinking Reagents

Cat #	MW	Storage	Soluble	Abs	EC	Em	Solvent	Notes
A-685	521.50	F,D,L	pH >6, DMF	491	78,000	515	pH 9	
A-20770	282.30	F,D,L	DMSO	<300		none		
B-1550	1019.45	FF,D	See Notes	<300		none		1
B-1616	1132.61	FF,D	See Notes	<300		none		1
B-10621	840.47	F,D,L	DMSO	549	88,000	575	MeOH	2
D-1379	350.01	L	DMF, MeCN	391	6,100	see Notes	MeOH	3
D-1532	154.24	D	H ₂ O	<300		none		
D-8451	396.35	D	pH >6	324	11,000	none	pH 8	4
E-2247	191.70	F,D	H ₂ O	<300		none		
H-2249	217.13	D	H ₂ O	<300		none		
M-1618	1012.40	FF,D	See Notes	<300		none		1
S-1531	312.36	F,D	DMF, MeCN	282	4,700	none	MeOH	5
S-1534	334.33	F,D	DMF, MeCN	<300		none		
S-1553	231.22	F,D	DMF, MeCN	<300		none		
T-2556	286.65	D	pH >5	<300		none		
T-6052	193.19	D	MeCN	<300		none		

For definitions of the contents of this data table, see "How to Use This Book" on page viii.

Notes

- Chloroform is the most generally useful solvent for preparing stock solutions of phospholipids (including sphingomyelins). Glycerophosphocholines are usually freely soluble in ethanol. Most other glycerophospholipids (phosphoethanolamines, phosphatidic acids and phosphoglycerols) are less soluble in ethanol, but solutions up to 1–2 mg/mL should be obtainable, using sonication to aid dispersion if necessary. Labeling of cells with fluorescent phospholipids can be enhanced by addition of cyclodextrins during incubation (J Biol Chem 274, 35359 (1999)).
- Iodoacetamides in solution undergo rapid photodecomposition to unreactive products. Minimize exposure to light prior to reaction.
- Bimanes are almost nonfluorescent until reacted with thiols. For monobromobimane conjugated to glutathione, Abs = 394 nm, Em = 490 nm (QY ~0.1–0.3) in pH 8 buffer (Methods Enzymol 143, 76 (1987); Methods Enzymol 251, 133 (1995)).
- D-8451 reaction product with thiols has Abs = 410 nm (EC = 14,000 cm⁻¹M⁻¹) (Methods Enzymol 233, 380 (1994)).
- After conjugation of S-1531, the degree of substitution can be determined by measuring the amount of 2-pyridinethione formed by treatment with DTT (D-1532) or TCEP (T-2556) from its absorbance at 343 nm (EC = 8000 cm⁻¹M⁻¹) (Biochem J 173, 723 (1978)).

Product List — 5.2 Chemical Crosslinking Reagents

Cat #	Product Name	Unit Size
A-685	5-((2-(and-3)-S-(acetylmercapto)succinoyl)amino)fluorescein (SAMSA fluorescein) *mixed isomers*	25 mg
A-20770	6-((acryloyl)amino)hexanoic acid, succinimidyl ester (acryloyl-X, SE)	5 mg
B-1616	<i>N</i> -((6-(biotinoyl)amino)hexanoyl)-1,2-dihexadecanoyl- <i>sn</i> -glycero-3-phosphoethanolamine, triethylammonium salt (biotin-X DHPE)	5 mg
B-1550	<i>N</i> -(biotinoyl)-1,2-dihexadecanoyl- <i>sn</i> -glycero-3-phosphoethanolamine, triethylammonium salt (biotin DHPE)	10 mg
B-10621	bis-((<i>N</i> -iodoacetyl)piperazinyl)sulfonerhodamine	5 mg
D-1379	dibromobimane (bBBr)	25 mg
D-8451	5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB; Ellman's reagent)	10 g
D-1532	dithiothreitol (DTT)	1 g
E-2247	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, hydrochloride (EDAC)	100 mg
H-2249	<i>N</i> -hydroxysulfosuccinimide, sodium salt (NHSS)	100 mg
M-1618	<i>N</i> -((4-maleimidylmethyl)cyclohexane-1-carbonyl)-1,2-dihexadecanoyl- <i>sn</i> -glycero-3-phosphoethanolamine, triethylammonium salt (MMCC DHPE)	5 mg
P-6305	Protein-Protein Crosslinking Kit *3 conjugations*	1 kit
S-1553	succinimidyl acetylthioacetate (SATA)	100 mg
S-1534	succinimidyl <i>trans</i> -4-(maleimidylmethyl)cyclohexane-1-carboxylate (SMCC)	100 mg
S-1531	succinimidyl 3-(2-pyridylthio)propionate (SPDP)	100 mg
T-6060	Thiol and Sulfide Quantitation Kit *50-250 assays*	1 kit
T-2556	tris-(2-carboxyethyl)phosphine, hydrochloride (TCEP)	1 g
T-6052	tris-(2-cyanoethyl)phosphine	1 g

Meeting Announcements

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